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(54) Title: MAMMALIAN INTERLEUKIN-10 HOMOLOGS: IL-D110 AND IL-D210

(57) Abstract: Purified genes encoding cytokine from a mammal, reagents related thereto including purified proteins, specific antibodies, and nucleic acids encoding these molecules are provided. Methods of using said reagents and diagnostic kits are also provided.

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MAMMALIAN INTERLEUKIN-10 HOMOLOGS: IL-D110 AND IL-D210

This application claims priority from U.S. Patent Application No. 09/322,806 filed May 27, 1999, which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention pertains to compositions related to proteins which function in controlling biology and physiology of mammalian cells, e.g., cells of a mammalian immune system. In particular, it provides purified genes, proteins, antibodies, and related reagents useful, e.g., to regulate activation, development, differentiation, and function of various cell types, including hematopoietic cells.

BACKGROUND OF THE INVENTION

Recombinant DNA technology refers generally to the technique of integrating genetic information from a donor source into vectors for subsequent processing, such as through introduction into a host, whereby the transferred genetic information is copied and/or expressed in the new environment. Commonly, the genetic information exists in the form of complementary DNA (cDNA) derived from messenger RNA (mRNA) coding for a desired protein product. The carrier is frequently a plasmid having the capacity to incorporate cDNA for later replication in a host and, in some cases, actually to control expression of the cDNA and thereby direct synthesis of the encoded product in the host.

For some time, it has been known that the mammalian immune response is based on a series of complex cellular interactions, called the "immune network". Recent research has provided new insights into the inner workings of this network. While it remains clear that much of the response does, in fact, revolve around the network-like interactions of lymphocytes, macrophages, granulocytes, and other cells, immunologists now generally hold the opinion that soluble

proteins, known as lymphokines, cytokines, or monokines, play a critical role in controlling these cellular interactions. Thus, there is considerable interest in the isolation, characterization, and mechanisms of action of cell modulatory factors, an understanding of which will lead to significant advancements in the diagnosis and therapy of numerous medical abnormalities, e.g., immune system disorders.

Lymphokines apparently mediate cellular activities in a variety of ways. They have been shown to support the proliferation, growth, and differentiation of pluripotential hematopoietic stem cells into vast numbers of progenitors comprising diverse cellular lineages making up a complex immune system. Proper and balanced interactions between the cellular components are necessary for a healthy immune response. The different cellular lineages often respond in a different manner when lymphokines are administered in conjunction with other agents.

Cell lineages especially important to the immune response include two classes of lymphocytes: B-cells, which can produce and secrete immunoglobulins (proteins with the capability of recognizing and binding to foreign matter to effect its removal), and T-cells of various subsets that secrete lymphokines and induce or suppress the B-cells and various other cells (including other T-cells) making up the immune network. These lymphocytes interact with many other cell types.

Another important cell lineage is the mast cell (which has not been positively identified in all mammalian species), which is a granule-containing connective tissue cell located proximal to capillaries throughout the body. These cells are found in especially high concentrations in the lungs, skin, and gastrointestinal and genitourinary tracts. Mast cells play a central role in allergy-related disorders, particularly anaphylaxis as follows: when selected antigens crosslink one class of immunoglobulins bound to receptors on the mast cell surface, the mast cell degranulates and releases mediators,

e.g., histamine, serotonin, heparin, and prostaglandins, which cause allergic reactions, e.g., anaphylaxis.

Research to better understand and treat various immune disorders has been hampered by the general inability to maintain cells of the immune system in vitro. Immunologists have discovered that culturing these cells can be accomplished through the use of T-cell and other cell supernatants, which contain various growth factors, including many of the lymphokines.

The gene encoding IL-10, originally designated Cytokine Synthesis Inhibitiory Factor (CSIF), was isolated in the 1980's. See, e.g., Mosmann, et al., U.S. Patent No. 5,231,012. Since then, much has been learned of the biology and physiology mediated by the cytokine. See, e.g., de Vries and de Waal Malefyt (1995) Interleukin-10 Landes Co., Austin, TX.

From the foregoing, it is evident that the discovery and development of new lymphokines, e.g., related to IL-10, could contribute to new therapies for a wide range of degenerative or abnormal conditions which directly or indirectly involve the immune system and/or hematopoietic cells. In particular, the discovery and development of lymphokines which enhance or potentiate the beneficial activities of known lymphokines would be highly advantageous. The present invention provides new interleukin compositions and related compounds, and methods for their use.

SUMMARY OF THE INVENTION

The present invention is directed to mammalian, e.g., rodent, canine, feline, primate, interleukin-10 homologs designated IL-D110 and IL-D210 and their biological activities. It includes nucleic acids coding for polypeptides themselves and methods for their production and use. The nucleic acids of the invention are characterized, in part, by their homology to described complementary DNA (cDNA) sequences enclosed herein, and/or by functional assays for IL-10-like activities applied to the polypeptides, which are typically

encoded by these nucleic acids. Methods for modulating or intervening in the control of an immune response are provided.

The invention provides an isolated or recombinant polynucleotide encoding an antigenic polypeptide comprising at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 1 or 4. Preferred embodiments include those which encode a mature polypeptide from SEQ ID NO: 2; hybridize at 55° C, less than 500 mM salt, and 50% formamide to the coding portions of SEQ ID NO: 1 or 4; or those where the temperature is at least 65° C; the salt is less than 200 mM; the temperature is at least 60° C and the salt is less than 300 mM; or which comprise at least 35 contiguous nucleotides of the coding portion of SEQ ID NO: 1 or 4. Another preferred embodiment is an expression vector comprising the polynucleotide. The invention further provides a host cell containing the expression vector, including a eukaryotic cell.

The invention also provides methods of making an antigenic polypeptide comprising expressing such a recombinant polynucleotides. Other methods include those for forming a duplex with such polynucleotides, e.g., comprising contacting the polynucleotide with a probe that hybridizes, under stringent conditions, to at least 25 contiguous nucleotides of the coding portion of SEQ ID NO: 1 or 4; thereby forming the duplex. To practice such methods are provided kits for the detection of such polynucleotides, comprising a polynucleotide that hybridizes, under stringent hybridization conditions, to at least 17 contiguous nucleotides of such a polynucleotide. Preferably, the probe is detectably labeled.

Binding compounds are provided, e.g., comprising an antibody binding site which specifically binds to: at least 17 contiguous amino acids from SEQ ID NO: 2 or 5; or a mature polypeptide from SEQ ID NO: 2 or 5. In certain embodiments, the antibody binding site is: specifically immunoreactive with a polypeptide of SEQ ID NO: 2 or 5; raised against a purified or recombinantly produced human IL-D110 or IL-D210 protein; or in a monoclonal antibody, Fab, or F(ab)2; or the binding compound is: an antibody molecule; a polyclonal antiserum; detectably labeled; sterile; or in a buffered composition.

Methods of using such binding compounds are provided, e.g., comprising contacting the binding compound with a biological sample comprising an antigen, wherein the contacting results in formation of a binding compound:antigen complex. Preferably, the biological sample is from a human, and the binding compound is an antibody.

Kits embodiments include those comprising the binding compound, and: instructional material for the use of the binding compound for the detection; or a compartment providing segregation of the binding compound.

Polypeptides are provided, including, e.g., a substantially pure or isolated antigenic polypeptide, which binds to the described binding composition, and further comprises at least 17 contiguous amino acids from SEQ ID NO: 2 or 5. In certain embodiments, the polypeptide further: comprises at least a fragment of at least 17 contiguous amino acid residues from a primate IL-D110 polypeptide; comprises at least a fragment of at least 17 contiguous amino acid residues from a primate IL-D210 polypeptide; is a soluble polypeptide; is detectably labeled; is in a sterile composition; is in a buffered composition; binds to a cell surface receptor; is recombinantly produced; or has a naturally occurring polypeptide sequence. A further embodiment is a polypeptide which comprises at least 25 contiguous amino acids of SEQ ID NO: 2 or 5.

The invention also provides methods of modulating physiology or development of a cell or tissue culture cells comprising contacting the cell with an agonist or antagonist of a primate IL-D110 or IL-D210. Preferably, the agonist is a mutein of the primate IL-D110 or IL-D210; the antagonist is an antibody which binds to the primate IL-D110 or IL-D210; the cell is a mesangial cell; or the modulating is inducing production of IL-D110 and the contacting is with the agonist.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

All references cited herein are incorporated herein by reference to the same extent as if each individual publication

or patent application was specifically and individually indicated to be incorporated by reference.

I. General

The present invention provides amino acid sequences and DNA sequences encoding various mammalian proteins which are cytokines, e.g., which are secreted molecules which can mediate a signal between immune or other cells. See, e.g., Paul (1998) Fundamental Immunology (4th ed.) Raven Press, N.Y. The full length cytokines, and fragments, or antagonists will be useful in physiological modulation of cells expressing a receptor. It is likely that IL-10 homologs, designated herein Interleukin DNAX designations 110 (IL-D110) and 210 (IL-D210), have either stimulatory or inhibitory effects on T-cells, B-cells, natural killer (NK) cells, macrophages, dendritic cells, hematopoietic progenitors, etc. The proteins will also be useful as antigens, e.g., immunogens, for raising antibodies to various epitopes on the protein, both linear and conformational epitopes.

A cDNA encoding IL-D110 was identified using motif searches from a sequence database. Likewise the IL-D210 was identified. Table 1 discloses the IL-D110 nucleotide and amino acid sequences. Table 2 discloses a reverse translation, or all of the nucleic acid sequences which encode the polypeptide using the universal genetic code. Tables 3 and 4 likewise describe the IL-D210. Table 5 shows an alignment of various IL-10 homologs.

Table 1: Primate, human, IL-D110 nucleotide and predicted amino-acid sequence. Coding sequence runs from about 59 to 598; see SEQ ID NO: 1 and 2. Predicted signal sequence indicated, but may be a few amino acids in either side, depending upon the cell.

```
atg gcc gcc ctg cag aaa tct gtg agc tct ttc ctt atg ggg acc ctg   106
Met Ala Ala Leu Gln Lys Ser Val Ser Ser Phe Leu Met Gly Thr Leu
           -30                 -25                 -20

gcc acc agc tgc ctc ctt ctc ttg gcc ctc ttg gta cag gga gga gca   154
Ala Thr Ser Cys Leu Leu Leu Leu Ala Leu Leu Val Gln Gly Gly Ala
        -15                 -10                  -5

gct gcg ccc atc agc tcc cac tgc agg ctt gac aag tcc aac ttc cag   202
Ala Ala Pro Ile Ser Ser His Cys Arg Leu Asp Lys Ser Asn Phe Gln
 -1   1              5                  10                  15
```

```
cag ccc tat atc acc aac cgc acc ttc atg ctg gct aag gag gnt agc   250
Gln Pro Tyr Ile Thr Asn Arg Thr Phe Met Leu Ala Lys Glu Xaa Ser
                 20                  25                  30

ttg gct gat aac aac aca gac gtt cgt ctc att ggg gag aaa ctg ttc   298
Leu Ala Asp Asn Asn Thr Asp Val Arg Leu Ile Gly Glu Lys Leu Phe
            35                  40                  45

cac gga gtc agt atg agt gag cgc tgc tat ctg atg aag cag gtg ctg   346
His Gly Val Ser Met Ser Glu Arg Cys Tyr Leu Met Lys Gln Val Leu
        50                  55                  60

aac ttc acc ctt gaa gaa gtg ctg ttc cct caa tct gat agg ttc cag   394
Asn Phe Thr Leu Glu Glu Val Leu Phe Pro Gln Ser Asp Arg Phe Gln
    65                  70                  75

cct tat atg cag gag gtg gtg ccc ttc ctg gcc agg ctc agc aac agg   442
Pro Tyr Met Gln Glu Val Val Pro Phe Leu Ala Arg Leu Ser Asn Arg
80                  85                  90                  95

cta agc aca tgt cat att gaa ggt gat gac ctg cat atc cag agg aat   490
Leu Ser Thr Cys His Ile Glu Gly Asp Asp Leu His Ile Gln Arg Asn
                100                 105                 110

gtg caa aag ctg aag gac aca gtg aaa aag ctt gga gag agt gga gag   538
Val Gln Lys Leu Lys Asp Thr Val Lys Lys Leu Gly Glu Ser Gly Glu
            115                 120                 125

atc aaa gca att gga gaa ctg gat ttg ctg ttt atg tct ctg aga aat   586
Ile Lys Ala Ile Gly Glu Leu Asp Leu Leu Phe Met Ser Leu Arg Asn
        130                 135                 140

gcc tgc att tgaccagagc aaagctgaaa aatgaataac taacccccctt          635
Ala Cys Ile
    145

tccctgctag aaataacaat tagatgcccc aaagcgattt tttttaacca aaaggaagat 695

gggaagccaa actccatcat gatgggtgga ttccaaatga acccctgcgt tagttacaaa 755

ggraaccaat gccacttttg tttataagac cagaaggtag actttctwag catagatatt 815

tattgataac atttcattgt aactggtgtt ctatacacag aaaacaattt atttttaaa 875

taattgtctt tttccataaa aaagattact ttccattcct ttaggggaaa aaacccctaa 935

atagcttcat gtttccataa tcagtacttt atatttataa atgtatttat tattattata 995

agactgcatt ttatttatat cattttatta atatggattt atttatagaa acatcattcg 1055

atattgctac ttgagtgtaa ggctaatatt gatatttatg acaataatta tagagctata 1115

acatgtttat ntgacctcaa tgaa                                        1139

MAALQKSVSSFLMGTLATSCLLLLALLVQGGAAAPISSHCRLDKSNFQQPYITNRTFMLAKEASLADNNTDVRLI
GEKLFHGVSMSERCYLMKQVLNFTLEEVLFPQSDRFQPYMQEVVPFLARLSNRLSTCHIEGDDLHIQRNVQKLKD
TVKKLGESGEIKAIGELDLLFMSLRNACI
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Table 2: Reverse Translation of the amino acid sequence of primate, e.g., human, IL-D110, e.g., those nucleotide sequences which encode said protein. See SEQ ID NO: 3.

ATGGCNGCNYTNCARAARWSNGTNWSNWSNTTYYTNATGGGNACNYTNGCNACNWSNTGYYTNYTNYTNYTNGCN
YTNYTNGTNCARGGNGGNGCNGCNGCNCCNATHWSNWSNCAYTGYMGNYTNGAYAARWSNAAYTTYCARCARCCN
TAYATHACNAAYMGNACNTTYATGYTNGCNAARGARGCNWSNYTNGCNGAYAAYAAYACNGAYGTNMGNYTNATH
GGNGARAARYTNTTYCAYGGNGTNWSNATGWSNGARMGNTGYTAYYTNATGAARCARGTNYTNAAYTTYACNYTN
GARGARGTNYTNTTYCCNCARWSNGAYMGNTTYCARCCNTAYATGCARGARGTNGTNCCNTTYYTNGCNMGNYTN
WSNAAYMGNYTNWSNACNTGYCAYATHGARGGNGAYGAYYTNCAYATHCARMGNAAYGTNCARAARYTNAARGAY
ACNGTNAARAARYTNGGNGARWSNGGNGARATHAARGCNATHGGNGARYTNGAYYTNYTNTTYATGWSNYTNMGN
AAYGCNTGYATH

Table 3: Primate, human, IL-D210 nucleotide and predicted amino-acid sequence. Coding region runs from about 3 to 308. See SEQ ID NO: 4 and 5.

```
ag cct gcg aat cga tgc tgc ctc ctg cgc cat ttg cta aga ctc tat    47
   Pro Ala Asn Arg Cys Cys Leu Leu Arg His Leu Leu Arg Leu Tyr
     1               5                  10                  15

ctg gac agg gta ttt aaa aac tac cag acc cct gac cat tat act ctc   95
Leu Asp Arg Val Phe Lys Asn Tyr Gln Thr Pro Asp His Tyr Thr Leu
                20                  25                  30

cgg aag atc agc agc ctc gcc aat tcc ttt ctt acc atc aag aag gac   143
Arg Lys Ile Ser Ser Leu Ala Asn Ser Phe Leu Thr Ile Lys Lys Asp
            35                  40                  45

ctc cgg ctc tgt cat gcc cac atg aca tgc cat tgt ggg gag gaa gca   191
Leu Arg Leu Cys His Ala His Met Thr Cys His Cys Gly Glu Glu Ala
        50                  55                  60

atg aag aaa tac agc cag att ctg agt cac ttt gaa aag ctg gaa cct   239
Met Lys Lys Tyr Ser Gln Ile Leu Ser His Phe Glu Lys Leu Glu Pro
    65                  70                  75

cag gca gca gtt gtg aag gct ttg ggg gaa cta gac att ctt ctg caa   287
Gln Ala Ala Val Val Lys Ala Leu Gly Glu Leu Asp Ile Leu Leu Gln
80                  85                  90                  95

tgg atg gag gag aca gaa taggaggaaa gtgatgctgc tgctaagaat          335
Trp Met Glu Glu Thr Glu
                100

attcgaggtc aagagctcca gtcttcaata cctgcagagg aggcatgacc ccaaaccacc 395

atctctttac tgtactagtc ttgtgctggt cacagtgtat cttatttatg cattacttgc 455

ttccttgcat gattgtcttt atgcatcccc aatcttaatt gngnccatac ttgtataaga 515

ttttt                                                             520

QPANRCCLLRHLLRLYLDRVFKNYQTPDHYTLRKISSLANSFLTIKKDLRLCHAHMTCHCGEEAMKKYSQILSHF
EKLEPQAAVVKALGELDILLQWMEETE
```

Table 4: Reverse translation of the amino acid sequence of primate, e.g., human, IL-D210, e.g., those nucleotide sequences which encode said protein. See SEQ ID NO: 6.

CARCCNGCNAAYMGNTGYTGYYTNYTNMGNCAYYTNYTNMGNYTNTAYYTNGAYMGNGTNTTYAARAAYTAYCAR
ACNCCNGAYCAYTAYACNYTNMGNAARATHWSNWSNYTNGCNAAYWSNTTYYTNACNATHAARAARGAYYTNMGN
YTNTGYCAYGCNCAYATGACNTGYCAYTGYGGNGARGARGCNATGAARAARTAYWSNCARATHYTNWSNCAYTTY
GARAARYTNGARCCNCARGCNGCNGTNGTNAARGCNYTNGGNGARYTNGAYATHYTNYTNCARTGGATGGARGAR
ACNGAR

Table 5: Comparison of various primate IL-10 homologs compared to IL-D110 and IL-D210. IL-10 is human IL-10 (SEQ ID NO: 7); AK155 is human IL-10 homolog (SEQ ID NO: 8; see USSN 08/934,959); IL-D19 is DNAX designation 19 (SEQ ID NO: 9); and IL-D20 is DNAX designation 20 (SEQ ID NO: 10).

```
IL-10       1     MHSSALLCCLVLLTGVRASPGQGTQ----S--ENSCTHFPGNLPNM  40
AK155       1     MLVNFILRCGLLLVTLSLAIAKHKQ----SSFTKSCYPRG----TL  38
IL-D110     1 MAALQKSVSSFLMGTLATSCLLLLALLVQGGAAAPISSHCRLDK----SN  46
IL-D210     1                                                     0
IL-D19      1   MKLQCVSLWLLGTILILCSVDNHG----------LRRCLIS-----TD  33
IL-D20      1     MQMVVLPCLGFTLLLWSQVSGAQGQ----EFHFGPCQVKG----VV  38


IL-10      41 LRDLRDAFSRVKTFFQMK--DQLDNLLLKE--SLLEDFKGYLGCQALSEM  86
AK155      39 SQAVDALYIKAAWLKATIPEDRIKNIRLLK--KKTKKQF-MKNCQFQEQL  85
IL-D110    47 FQQPYITNRTFMLAKEXSLADNNTDVRLIG-EKLFHGVSMSERCYLMKQV  95
IL-D210     1                                          PANRCCLLRHL  11
IL-D19     34 MHHIEESFQEIKRAIQAK--DTFPNVTILSTLETLQIIKPLDVCCVTKNL  81
IL-D20     39 PQKLWEAFWAVKDTMQAQ--DNITSARLLQ-QEVLQNVSDAESCYLVHTL  85
                                                      *     .


IL-10      87 IQFYLEEVMPQAE--NQDPDIKAHVNSLGENLKTLRLRLRRCHR---FLP 131
AK155      86 LSFFMEDVFGQLQ-----LQGCKKIR-FVEDFHSLRQKLSHCIS---CAS 126
IL-D110    96 LNFTLEEVLFPQS-----DRFQPYMQEVVPFLARLSNRLSTCHIE---GD 137
IL_D210    12 LRLYLDRVFKNYQ--TPDHYTLRKISSLANSFLTIKKDLRLCHAHMTCHC  59
IL-D19     82 LAFYVDRVFKDHQ--EPNPKILRKISSIANSFLYMQKTLRQCQEQRQCHC 129
IL-D20     86 LEFYLKTVFKNYHNRTVEVRTLKSFSTLANNFVLIVSQLQPSQENEMFSI 135
              .   .  *                          .   *  .


IL-10     132 CENKSKAVEQVKNAFNKLQ-EKGIYKAMSEFDIFINYIEAYMTMKIRN 178
AK155     127 SAREMKSITRMKRIFYRIG-NKGIYKAISELDILLSWIKKLLESSQ   171
IL-D110   138 DLHIQRNVQKLKDTVKKLG-ESGEIKAIGELDLLFMSLRNACI      179
IL-D210    60 GEEAMKKYSQILSHFEKLEPQAAVVKALGELDILLQWMEETE       101
IL-19     130 RQEATNATRVIHDNYDQLEVHAAAIKSLGELDVFLAWINKNHEVMSSA 177
IL-20     136 RDSAHRRFLLFRRAFKQLDVEAALTKALGEVDILLTWMQKFYKL     179
                             ..       *.. * *.    .
```

The structural homologies of IL-D110 and IL-D210 (collectively referred to as IL-D10s) to the related IL-10 proteins suggest similar functions of these molecules. IL-D110 and IL-D210, as small chain cytokines, likely mediate immune functions via receptors of the class of cytokine receptors, possibly even sharing parts or all of the functional IL-10 receptor complex.

IL-D10 agonists, or antagonists, may also act as functional or receptor antagonists, e.g., which block IL-10 binding to its receptor, or mediating the opposite actions. Thus, IL-D10, or its antagonists, may be useful in the treatment of abnormal immune disorders, e.g., T cell immune deficiencies, chronic inflammation, or tissue rejection.

The natural antigens are capable of mediating various biochemical responses which lead to biological or physiological responses in target cells. The embodiments characterized herein are from human, but other primate, or other species counterparts are expected to exist in nature. Additional sequences for proteins in other mammalian species, e.g., primates, canines, felines, and rodents, should also be available. See below. The descriptions below are directed, for exemplary purposes, to a human IL-D10, but are likewise applicable to related embodiments from other species.

II. Purified IL-D10

Primate, e.g., human, IL-D110 amino acid sequence is shown in SEQ ID NO: 2; and IL-D210 described in SEQ ID NO: 5. These amino acid sequences, provided amino to carboxy, are important in providing sequence information on the cytokines allowing for distinguishing the protein antigens from other proteins and exemplifying numerous variants. Moreover, the peptide sequences allow preparation of peptides to generate antibodies to recognize such segments, and nucleotide sequences allow preparation of oligonucleotide probes, both of which are strategies for detection or isolation, e.g., cloning, of genes encoding such sequences. Alternatively, the expression of nucleic acid or protein may correlate with significant medical problems.

As used herein, the term "human IL-D110" shall encompass, when used in a protein context, a protein having amino acid sequence shown in SEQ ID NO: 2, or a significant fragment of such a protein. Binding components, e.g., antibodies, typically bind to an IL-D110 with high affinity, e.g., at least about 100 nM, usually better than about 30 nM, preferably better than about 10 nM, and more preferably at

better than about 3 nM. Homologous proteins would be found in mammalian species other than human, e.g., other primates or rodents. Non-mammalian species should also possess structurally or functionally related genes and proteins, e.g., birds or amphibians.

The term "polypeptide" as used herein includes a significant fragment or segment, and encompasses a stretch of amino acid residues of at least about 8 amino acids, generally at least about 12 amino acids, typically at least about 16 amino acids, preferably at least about 20 amino acids, and, in particularly preferred embodiments, at least about 30 or more amino acids, e.g., 35, 40, 45, 50, etc. Such fragments may have ends which begin and/or end at virtually all positions, e.g., beginning at residues 1, 2, 3, etc., and ending at, e.g., 150, 149, 148, etc., in all combinations.

Based upon similarity to interferon-γ, the IL-D10s are likely to assume a structure with similar helix structure. Predicted boundaries for domain boundaries, e.g., helices A, B, C, D, E, and/or F, can be made. See Reineke, et al. (1998) Protein Science 7:951-960; Walter and Nagabhushan (1995) Biochemistry 34:12118-12125; Walter, et al. (1995) Nature 376:230-235; Zdanov, et al. (1995) Structure 34:591-601; and Zdanov, et al. (1996) Protein Science 5:1955-1962. Particularly interesting peptides would contain intact sequence from structural domain boundaries, e.g., helices A, B, C, D, E, and/or F. See Table 5. For IL-D110, the predicted helix A would run about over SNF...LAD; helix B from GEK...VSM; helix C from ERC to QSD; helix D from FQP...STC; helix E from QRN...KLG; and helix F from ESG...ACI. Likewise, for IL-D210, the predicted helix C would run about over NRC...YQT; helix D over TLR...RLC; helix E over MKK...KLE; and helix F over QAA...ETE.

The term "binding composition" refers to molecules that bind with specificity to IL-D10s, e.g., in an antibody-antigen interaction. It also includes compounds, e.g., proteins, which specifically associate with each IL-D10, including in a natural physiologically relevant protein-protein interaction, either covalent or non-covalent. The molecule may be a

polymer, or chemical reagent. A functional analog may be a protein with structural modifications, or it may be a molecule which has a molecular shape which interacts with the appropriate binding determinants. The compounds may serve as agonists or antagonists of a receptor binding interaction, see, e.g., Goodman, et al. (eds. 1990) Goodman & Gilman's: The Pharmacological Bases of Therapeutics (8th ed.), Pergamon Press.

Substantially pure typically means that the protein is free from other contaminating proteins, nucleic acids, or other biologicals derived from the original source organism. Purity may be assayed by standard methods, typically by weight, and will ordinarily be at least about 40% pure, generally at least about 50% pure, often at least about 60% pure, typically at least about 80% pure, preferably at least about 90% pure, and in most preferred embodiments, at least about 95% pure. Carriers or excipients will often be added.

Solubility of a polypeptide or fragment depends upon the environment and the polypeptide. Many parameters affect polypeptide solubility, including temperature, electrolyte environment, size and molecular characteristics of the polypeptide, and nature of the solvent. Typically, the temperature at which the polypeptide is used ranges from about 4° C to about 65° C. Usually the temperature at use is greater than about 18° C. For diagnostic purposes, the temperature will usually be about room temperature or warmer, but less than the denaturation temperature of components in the assay. For therapeutic purposes, the temperature will usually be body temperature, typically about 37° C for humans and mice, though under certain situations the temperature may be raised or lowered in situ or in vitro.

The size and structure of the polypeptide should generally be in a substantially stable state, and usually not in a denatured state. The polypeptide may be associated with other polypeptides in a quaternary structure, e.g., to confer solubility, or associated with lipids or detergents.

The solvent and electrolytes will usually be a biologically compatible buffer, of a type used for

preservation of biological activities, and will usually approximate a physiological aqueous solvent. Usually the solvent will have a neutral pH, typically between about 5 and 10, and preferably about 7.5. On some occasions, one or more detergents will be added, typically a mild non-denaturing one, e.g., CHS (cholesteryl hemisuccinate) or CHAPS (3-[3-cholamidopropyl)dimethylammonio]-1-propane sulfonate), or a low enough concentration as to avoid significant disruption of structural or physiological properties of the protein.

III. Physical Variants

This invention also encompasses proteins or peptides having substantial amino acid sequence identity with the amino acid sequence of each of the IL-D10 antigens. The variants include species, polymorphic, or allelic variants.

Amino acid sequence homology, or sequence identity, is determined by optimizing residue matches, if necessary, by introducing gaps as required. See also Needleham, et al. (1970) J. Mol. Biol. 48:443-453; Sankoff, et al. (1983) Chapter One in Time Warps, String Edits, and Macromolecules: The Theory and Practice of Sequence Comparison, Addison-Wesley, Reading, MA; and software packages from IntelliGenetics, Mountain View, CA; and the University of Wisconsin Genetics Computer Group, Madison, WI. Sequence identity changes when considering conservative substitutions as matches. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. The conservation may apply to biological features, functional features, or structural features. Homologous amino acid sequences are typically intended to include natural polymorphic or allelic and interspecies variations in each respective protein sequence. Typical homologous proteins or peptides will have from 25-100% identity (if gaps can be introduced), to 50-100% identity (if conservative substitutions are included) with the amino acid sequence of the IL-D10s. Identity measures will be at least

about 35%, generally at least about 40%, often at least about 50%, typically at least about 60%, usually at least about 70%, preferably at least about 80%, and more preferably at least about 90%.

An isolated IL-D10 DNA can be readily modified by nucleotide substitutions, nucleotide deletions, nucleotide insertions, and inversions of nucleotide stretches. These modifications result in novel DNA sequences which encode these antigens, their derivatives, or proteins having similar physiological, immunogenic, antigenic, or other functional activity. These modified sequences can be used to produce mutant antigens or to enhance expression. Enhanced expression may involve gene amplification, increased transcription, increased translation, and other mechanisms. "Mutant IL-D10" encompasses a polypeptide otherwise falling within the sequence identity definition of the IL-D10 as set forth above, but having an amino acid sequence which differs from that of IL-D10 as normally found in nature, whether by way of deletion, substitution, or insertion. This generally includes proteins having significant identity with a protein having sequence of SEQ ID NO: 2 or 5, and as sharing various biological activities, e.g., antigenic or immunogenic, with those sequences, and in preferred embodiments contain most of the full length disclosed sequences. Full length sequences will typically be preferred, though truncated versions will also be useful, likewise, genes or proteins found from natural sources are typically most desired. Similar concepts apply to different IL-D110 or IL-D210 proteins, particularly those found in various warm blooded animals, e.g., mammals and birds. These descriptions are generally meant to encompass all IL-D10 proteins, not limited to the particular embodiments specifically discussed.

IL-D10 mutagenesis can also be conducted by making amino acid insertions or deletions. Substitutions, deletions, insertions, or any combinations may be generated to arrive at a final construct. Insertions include amino- or carboxy-terminal fusions. Random mutagenesis can be conducted at a target codon and the expressed mutants can then be screened

for the desired activity. Methods for making substitution mutations at predetermined sites in DNA having a known sequence are well known in the art, e.g., by M13 primer mutagenesis or polymerase chain reaction (PCR) techniques. See, e.g., Sambrook, et al. (1989); Ausubel, et al. (1987 and Supplements); and Kunkel, et al. (1987) Methods in Enzymol. 154:367-382.

The present invention also provides recombinant proteins, e.g., heterologous fusion proteins using segments from these proteins. A heterologous fusion protein is a fusion of proteins or segments which are naturally not normally fused in the same manner. A similar concept applies to heterologous nucleic acid sequences.

In addition, new constructs may be made from combining similar functional domains from other proteins. For example, target-binding or other segments may be "swapped" between different new fusion polypeptides or fragments. See, e.g., Cunningham, et al. (1989) Science 243:1330-1336; and O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992.

The phosphoramidite method described by Beaucage and Carruthers (1981) Tetra. Letts. 22:1859-1862, will produce suitable synthetic DNA fragments. A double stranded fragment will often be obtained either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence, e.g., PCR techniques.

IV. Functional Variants

The blocking of physiological response to IL-D10s may result from the competitive inhibition of binding of the ligand to its respective receptor. IL-D10 binding to IL-10 receptor may serve to induce signaling, e.g., send a signal similar to binding by IL-10. Alternatively, IL-D10 binding to IL-10 receptor may block IL-10 signaling. An IL-D10 antagonist would be expected to have the opposite effect as IL-D10.

In vitro assays of the present invention will often use isolated protein, soluble fragments comprising receptor binding segments of these proteins, or fragments attached to solid phase substrates. These assays will also allow for the diagnostic determination of the effects of either binding segment mutations and modifications, or cytokine mutations and modifications, e.g., IL-D10 analogs.

This invention also contemplates the use of competitive drug screening assays, e.g., where neutralizing antibodies to the cytokine, or receptor binding fragments compete with a test compound.

"Derivatives" of IL-D110 or IL-D210 antigens include amino acid sequence mutants from naturally occurring forms, glycosylation variants, and covalent or aggregate conjugates with other chemical moieties. Covalent derivatives can be prepared by linkage of functionalities to groups which are found in IL-D10 amino acid side chains or at the N- or C-termini, e.g., by standard means. See, e.g., Lundblad and Noyes (1988) Chemical Reagents for Protein Modification, vols. 1-2, CRC Press, Inc., Boca Raton, FL; Hugli (ed. 1989) Techniques in Protein Chemistry, Academic Press, San Diego, CA; and Wong (1991) Chemistry of Protein Conjugation and Cross Linking, CRC Press, Boca Raton, FL.

In particular, glycosylation alterations are included, e.g., made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing, or in further processing steps. See, e.g., Elbein (1987) Ann. Rev. Biochem. 56:497-534. Also embraced are versions of the peptides with the same primary amino acid sequence which have other minor modifications, including phosphorylated amino acid residues, e.g., phosphotyrosine, phosphoserine, or phosphothreonine.

Fusion polypeptides between IL-D10s and other homologous or heterologous proteins are also provided. Many cytokine receptors or other surface proteins are multimeric, e.g., homodimeric entities, and a repeat construct may have various advantages, including lessened susceptibility to proteolytic cleavage. Typical examples are fusions of a reporter polypeptide, e.g., luciferase, with a segment or domain of a

protein, e.g., a receptor-binding segment, so that the presence or location of the fused ligand may be easily determined. See, e.g., Dull, et al., U.S. Patent No. 4,859,609. Other gene fusion partners include bacterial ß-galactosidase, trpE, Protein A, ß-lactamase, alpha amylase, alcohol dehydrogenase, yeast alpha mating factor, and detection or purification tags such as a FLAG sequence of His6 sequence. See, e.g., Godowski, et al. (1988) Science 241:812-816.

Fusion peptides will typically be made by either recombinant nucleic acid methods or by synthetic polypeptide methods. Techniques for nucleic acid manipulation and expression are described generally, e.g., in Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, Cold Spring Harbor Laboratory; and Ausubel, et al. (eds. 1993) Current Protocols in Molecular Biology, Greene and Wiley, NY. Techniques for synthesis of polypeptides are described, e.g., in Merrifield (1963) J. Amer. Chem. Soc. 85:2149-2156; Merrifield (1986) Science 232: 341-347; Atherton, et al. (1989) Solid Phase Peptide Synthesis: A Practical Approach, IRL Press, Oxford; and Grant (1992) Synthetic Peptides: A User's Guide, W.H. Freeman, NY. Refolding methods may be applicable to synthetic proteins.

This invention also contemplates the use of derivatives of IL-D10 proteins other than variations in amino acid sequence or glycosylation. Such derivatives may involve covalent or aggregative association with chemical moieties or protein carriers. Covalent or aggregative derivatives will be useful as immunogens, as reagents in immunoassays, or in purification methods such as for affinity purification of binding partners, e.g., other antigens. An IL-D10 can be immobilized by covalent bonding to a solid support such as cyanogen bromide-activated SEPHAROSE, by methods which are well known in the art, or adsorbed onto polyolefin surfaces, with or without glutaraldehyde cross-linking, for use in the assay or purification of anti-IL-D10 antibodies or an alternative binding composition. The IL-D10 proteins can also be labeled with a detectable group, e.g., for use in

diagnostic assays. Purification of IL-D110 or IL-D210 may be effected by an immobilized antibody or complementary binding partner, e.g., binding portion of a receptor.

A solubilized IL-D10 or fragment of this invention can be used as an immunogen for the production of antisera or antibodies specific for binding. Purified antigen can be used to screen monoclonal antibodies or antigen-binding fragments, encompassing antigen binding fragments of natural antibodies, e.g., Fab, Fab', $F(ab)_2$, etc. Purified IL-D10 antigens can also be used as a reagent to detect antibodies generated in response to the presence of elevated levels of the cytokine, which may be diagnostic of an abnormal or specific physiological or disease condition. This invention contemplates antibodies raised against amino acid sequences encoded by nucleotide sequence shown in SEQ ID NO: 1 or 4, or fragments of proteins containing it. In particular, this invention contemplates antibodies having binding affinity to or being raised against specific domains, e.g., helices A, B, C, D, E, or F.

The present invention contemplates the isolation of additional closely related species variants. Southern and Northern blot analysis will establish that similar genetic entities exist in other mammals. It is likely that IL-D10s are widespread in species variants, e.g., rodents, lagomorphs, carnivores, artiodactyla, perissodactyla, and primates.

The invention also provides means to isolate a group of related antigens displaying both distinctness and similarities in structure, expression, and function. Elucidation of many of the physiological effects of the molecules will be greatly accelerated by the isolation and characterization of additional distinct species or polymorphic variants of them. In particular, the present invention provides useful probes for identifying additional homologous genetic entities in different species.

The isolated genes will allow transformation of cells lacking expression of IL-D110 or IL-D210, e.g., either species types or cells which lack corresponding proteins and exhibit negative background activity. This should allow analysis of

the function of IL-D10s in comparison to untransformed control cells.

Dissection of critical structural elements which effect the various physiological functions mediated through these antigens is possible using standard techniques of modern molecular biology, particularly in comparing members of the related class. See, e.g., the homolog-scanning mutagenesis technique described in Cunningham, et al. (1989) Science 243:1339-1336; and approaches used in O'Dowd, et al. (1988) J. Biol. Chem. 263:15985-15992; and Lechleiter, et al. (1990) EMBO J. 9:4381-4390.

Intracellular functions would probably involve receptor signaling. However, protein internalization may occur under certain circumstances, and interaction between intracellular components and cytokine may occur. Specific segments of interaction of IL-110 or IL-D210 with interacting components may be identified by mutagenesis or direct biochemical means, e.g., cross-linking or affinity methods. Structural analysis by crystallographic or other physical methods will also be applicable. Further investigation of the mechanism of signal transduction will include study of associated components which may be isolatable by affinity methods or by genetic means, e.g., complementation analysis of mutants.

Further study of the expression and control of IL-D10 will be pursued. The controlling elements associated with the antigens should exhibit differential physiological, developmental, tissue specific, or other expression patterns. Upstream or downstream genetic regions, e.g., control elements, are of interest.

Structural studies of the IL-D10 antigens will lead to design of new antigens, particularly analogs exhibiting agonist or antagonist properties on the molecule. This can be combined with previously described screening methods to isolate antigens exhibiting desired spectra of activities.

V. Antibodies

Antibodies can be raised to various epitopes of the IL-D110 or IL-D210 proteins, including species, polymorphic,

or allelic variants, and fragments thereof, both in their naturally occurring forms and in their recombinant forms. Additionally, antibodies can be raised to IL-D10s in either their active forms or in their inactive forms, including native or denatured versions. Anti-idiotypic antibodies are also contemplated.

Antibodies, including binding fragments and single chain versions, against predetermined fragments of the antigens can be raised by immunization of animals with conjugates of the fragments with immunogenic proteins. Monoclonal antibodies are prepared from cells secreting the desired antibody. These antibodies can be screened for binding to normal or defective IL-D110 or IL-D210, or screened for agonistic or antagonistic activity, e.g., mediated through a receptor. Antibodies may be agonistic or antagonistic, e.g., by sterically blocking binding to a receptor. These monoclonal antibodies will usually bind with at least a K_D of about 1 mM, more usually at least about 300 μM, typically at least about 100 μM, more typically at least about 30 μM, preferably at least about 10 μM, and more preferably at least about 3 μM or better.

The antibodies of this invention can also be useful in diagnostic applications. As capture or non-neutralizing antibodies, they can be screened for ability to bind to the antigens without inhibiting binding to a receptor. As neutralizing antibodies, they can be useful in competitive binding assays. They will also be useful in detecting or quantifying IL-D10 protein or its receptors. See, e.g., Chan (ed. 1987) Immunology: A Practical Guide, Academic Press, Orlando, FLA; Price and Newman (eds. 1991) Principles and Practice of Immunoassay, Stockton Press, N.Y.; and Ngo (ed. 1988) Nonisotopic Immunoassay, Plenum Press, N.Y. Cross absorptions or other tests will identify antibodies which exhibit various spectra of specificities, e.g., unique or shared species specificities.

Further, the antibodies, including antigen binding fragments, of this invention can be potent antagonists that bind to the antigen and inhibit functional binding, e.g., to a receptor which may elicit a biological response. They also

can be useful as non-neutralizing antibodies and can be coupled to toxins or radionuclides so that when the antibody binds to antigen, a cell expressing it, e.g., on its surface, is killed. Further, these antibodies can be conjugated to drugs or other therapeutic agents, either directly or indirectly by means of a linker, and may effect drug targeting.

Antigen fragments may be joined to other materials, particularly polypeptides, as fused or covalently joined polypeptides to be used as immunogens. An antigen and its fragments may be fused or covalently linked to a variety of immunogens, such as keyhole limpet hemocyanin, bovine serum albumin, tetanus toxoid, etc. See Microbiology, Hoeber Medical Division, Harper and Row, 1969; Landsteiner (1962) Specificity of Serological Reactions, Dover Publications, New York; Williams, et al. (1967) Methods in Immunology and Immunochemistry, vol. 1, Academic Press, New York; and Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press, NY, for descriptions of methods of preparing polyclonal antisera.

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies may be found in, e.g., Stites, et al. (eds.) Basic and Clinical Immunology (4th ed.), Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane (1988) Antibodies: A Laboratory Manual, CSH Press; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed.), Academic Press, New York; and particularly in Kohler and Milstein (1975) in Nature 256:495-497, which discusses one method of generating monoclonal antibodies.

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors. See, Huse, et al. (1989) "Generation of a Large Combinatorial Library of the Immunoglobulin Repertoire in Phage Lambda," Science 246:1275-1281; and Ward, et al. (1989)

Nature 341:544-546. The polypeptides and antibodies of the present invention may be used with or without modification, including chimeric or humanized antibodies. Frequently, the polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents, teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced, see Cabilly, U.S. Patent No. 4,816,567; Moore, et al., U.S. Patent No. 4,642,334; and Queen, et al. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033.

The antibodies of this invention can also be used for affinity chromatography in isolating the protein. Columns can be prepared where the antibodies are linked to a solid support. See, e.g., Wilchek et al. (1984) Meth. Enzymol. 104:3-55.

Antibodies raised against each IL-D110 or IL-210 will also be useful to raise anti-idiotypic antibodies. These will be useful in detecting or diagnosing various immunological conditions related to expression of the respective antigens.

VI. Nucleic Acids

The described peptide sequences and the related reagents are useful in detecting, isolating, or identifying a DNA clone encoding IL-D110 or IL-D210, e.g., from a natural source. Typically, it will be useful in isolating a gene from mammal, and similar procedures will be applied to isolate genes from other species, e.g., warm blooded animals, such as birds and mammals. Cross hybridization will allow isolation of IL-D10 from the same, e.g., polymorphic variants, or other species. A number of different approaches should be available to successfully isolate a suitable nucleic acid clone.

The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein can be presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual, Cold Spring Harbor Press.

For example, the specific binding composition could be used for screening of an expression library made from a cell line which expresses an IL-D10. Screening of intracellular expression can be performed by various staining or immunofluorescence procedures. Binding compositions could be used to affinity purify or sort out cells expressing a surface fusion protein.

The peptide segments can also be used to predict appropriate oligonucleotides to screen a library. The genetic code can be used to select appropriate oligonucleotides useful as probes for screening. See, e.g., SEQ ID NO: 1 or 4. In combination with polymerase chain reaction (PCR) techniques, synthetic oligonucleotides will be useful in selecting correct clones from a library. Complementary sequences will also be used as probes, primers, or antisense strands. Various fragments should be particularly useful, e.g., coupled with anchored vector or poly-A complementary PCR techniques or with complementary DNA of other peptides.

This invention contemplates use of isolated DNA or fragments to encode a biologically active corresponding IL-D110 or IL-D210 polypeptide. In addition, this invention covers isolated or recombinant DNA which encodes a biologically active protein or polypeptide and which is capable of hybridizing under appropriate conditions with the DNA sequences described herein. Said biologically active protein or polypeptide can be an intact antigen, or fragment, and have an amino acid sequence disclosed in, e.g., SEQ ID NO: 2 or 5. Further, this invention covers the use of isolated or recombinant DNA, or fragments thereof, which encode proteins which exhibit high identity to an IL-D10 or which was isolated using cDNA encoding an IL-D10 as a probe. The isolated DNA

can have the respective regulatory sequences in the 5' and 3' flanks, e.g., promoters, enhancers, poly-A addition signals, and others.

An "isolated" nucleic acid is a nucleic acid, e.g., an RNA, DNA, or a mixed polymer, which is substantially separated from other components which naturally accompany a native sequence, e.g., ribosomes, polymerases, and/or flanking genomic sequences from the originating species. The term embraces a nucleic acid sequence which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems. A substantially pure molecule includes isolated forms of the molecule. Generally, the nucleic acid will be in a vector or fragment less than about 50 kb, usually less than about 30 kb, typically less than about 10 kb, and preferably less than about 6 kb.

An isolated nucleic acid will generally be a homogeneous composition of molecules, but will, in some embodiments, contain minor heterogeneity. This heterogeneity is typically found at the polymer ends or portions not critical to a desired biological function or activity.

A "recombinant" nucleic acid is defined either by its method of production or its structure. In reference to its method of production, e.g., a product made by a process, the process is use of recombinant nucleic acid techniques, e.g., involving human intervention in the nucleotide sequence, typically selection or production. Alternatively, it can be a nucleic acid made by generating a sequence comprising fusion of two fragments which are not naturally contiguous to each other, but is meant to exclude products of nature, e.g., naturally occurring mutants. Thus, e.g., products made by transforming cells with any unnaturally occurring vector is encompassed, as are nucleic acids comprising sequence derived using any synthetic oligonucleotide process. Such is often done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site.

Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a single genetic entity comprising a desired combination of functions not found in the commonly available natural forms. Restriction enzyme recognition sites are often the target of such artificial manipulations, but other site specific targets, e.g., promoters, DNA replication sites, regulation sequences, control sequences, or other useful features may be incorporated by design. A similar concept is intended for a recombinant, e.g., fusion, polypeptide. Specifically included are synthetic nucleic acids which, by genetic code redundancy, encode polypeptides similar to fragments of these antigens, and fusions of sequences from various different species or polymorphic variants.

A significant "fragment" in a nucleic acid context is a contiguous segment of at least about 17 nucleotides, generally at least about 22 nucleotides, ordinarily at least about 29 nucleotides, more often at least about 35 nucleotides, typically at least about 41 nucleotides, usually at least about 47 nucleotides, preferably at least about 55 nucleotides, and in particularly preferred embodiments will be at least about 60 or more nucleotides, e.g., 67, 73, 81, 89, 95, etc.

A DNA which codes for an IL-D110 or IL-D210 protein will be particularly useful to identify genes, mRNA, and cDNA species which code for related or similar proteins, as well as DNAs which code for homologous proteins from different species. There are likely homologs in other species, including primates, rodents, canines, felines, and birds. Various IL-D10 proteins should be homologous and are encompassed herein. However, even proteins that have a more distant evolutionary relationship to the antigen can readily be isolated under appropriate conditions using these sequences if they are sufficiently homologous. Primate IL-D10 proteins are of particular interest.

Recombinant clones derived from the genomic sequences, e.g., containing introns, will be useful for transgenic studies, including, e.g., transgenic cells and organisms, and

for gene therapy. See, e.g., Goodnow (1992) "Transgenic Animals" in Roitt (ed.) Encyclopedia of Immunology, Academic Press, San Diego, pp. 1502-1504; Travis (1992) Science 256:1392-1394; Kuhn, et al. (1991) Science 254:707-710; Capecchi (1989) Science 244:1288; Robertson (ed. 1987) Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, IRL Press, Oxford; and Rosenberg (1992) J. Clinical Oncology 10:180-199.

Substantial homology, e.g., identity, in the nucleic acid sequence comparison context means either that the segments, or their complementary strands, when compared, are identical when optimally aligned, with appropriate nucleotide insertions or deletions, in at least about 50% of the nucleotides, generally at least about 58%, ordinarily at least about 65%, often at least about 71%, typically at least about 77%, usually at least about 85%, preferably at least about 95 to 98% or more, and in particular embodiments, as high as about 99% or more of the nucleotides. Alternatively, substantial homology exists when the segments will hybridize under selective hybridization conditions, to a strand, or its complement, typically using a sequence of IL-D110 or IL-D210, e.g., in SEQ ID NO: 1 or 4. Typically, selective hybridization will occur when there is at least about 55% identity over a stretch of at least about 30 nucleotides, preferably at least about 75% over a stretch of about 25 nucleotides, and most preferably at least about 90% over about 20 nucleotides. See, Kanehisa (1984) Nuc. Acids Res. 12:203-213. The length of identity comparison, as described, may be over longer stretches, and in certain embodiments will be over a stretch of at least about 17 nucleotides, usually at least about 28 nucleotides, typically at least about 40 nucleotides, and preferably at least about 75 to 100 or more nucleotides.

Stringent conditions, in referring to homology in the hybridization context, will be stringent combined conditions of salt, temperature, organic solvents, and other parameters, typically those controlled in hybridization reactions. Stringent temperature conditions will usually include temperatures in excess of about 30° C, usually in excess of

about 37° C, typically in excess of about 55° C, preferably in excess of about 70° C. Stringent salt conditions will ordinarily be less than about 1000 mM, usually less than about 400 mM, typically less than about 250 mM, preferably less than about 150 mM, including about 100, 50, or even 20 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur and Davidson (1968) J. Mol. Biol. 31:349-370.

IL-D10s from other mammalian species can be cloned and isolated by cross-species hybridization of closely related species. Homology may be relatively low between distantly related species, and thus hybridization of relatively closely related species is advisable. Alternatively, preparation of an antibody preparation which exhibits less species specificity may be useful in expression cloning approaches.

VII. Making IL-D110 or IL-D210; Mimetics

DNA which encodes the IL-D10 or fragments thereof can be obtained by chemical synthesis, screening cDNA libraries, or screening genomic libraries prepared from a wide variety of cell lines or tissue samples. See, e.g., Okayama and Berg (1982) Mol. Cell. Biol. 2:161-170; Gubler and Hoffman (1983) Gene 25:263-269; and Glover (ed. 1984) DNA Cloning: A Practical Approach, IRL Press, Oxford. Alternatively, the sequences provided herein provide useful PCR primers or allow synthetic or other preparation of suitable genes encoding an IL-D10; including naturally occurring embodiments.

This DNA can be expressed in a wide variety of host cells for the synthesis of a full-length IL-D10 or fragments which can in turn, e.g., be used to generate polyclonal or monoclonal antibodies; for binding studies; for construction and expression of modified molecules; and for structure/function studies.

Vectors, as used herein, comprise plasmids, viruses, bacteriophage, integratable DNA fragments, and other vehicles which enable the integration of DNA fragments into the genome of the host. See, e.g., Pouwels, et al. (1985 and Supplements) Cloning Vectors: A Laboratory Manual, Elsevier,

N.Y.; and Rodriguez, et al. (1988)(eds.) Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Buttersworth, Boston, MA.

For purposes of this invention, DNA sequences are operably linked when they are functionally related to each other. For example, DNA for a presequence or secretory leader is operably linked to a polypeptide if it is expressed as a preprotein or participates in directing the polypeptide to the cell membrane or in secretion of the polypeptide. A promoter is operably linked to a coding sequence if it controls the transcription of the polypeptide; a ribosome binding site is operably linked to a coding sequence if it is positioned to permit translation. Usually, operably linked means contiguous and in reading frame, however, certain genetic elements such as repressor genes are not contiguously linked but still bind to operator sequences that in turn control expression. See, e.g., Rodriguez, et al., Chapter 10, pp. 205-236; Balbas and Bolivar (1990) Methods in Enzymology 185:14-37; and Ausubel, et al. (1993) Current Protocols in Molecular Biology, Greene and Wiley, NY.

Representative examples of suitable expression vectors include pCDNA1; pCD, see Okayama, et al. (1985) Mol. Cell Biol. 5:1136-1142; pMC1neo Poly-A, see Thomas, et al. (1987) Cell 51:503-512; and a baculovirus vector such as pAC 373 or pAC 610. See, e.g., Miller (1988) Ann. Rev. Microbiol. 42:177-199.

It will often be desired to express an IL-D10 polypeptide in a system which provides a specific or defined glycosylation pattern. See, e.g., Luckow and Summers (1988) Bio/Technology 6:47-55; and Kaufman (1990) Meth. Enzymol. 185:487-511.

The IL-D10, or a fragment thereof, may be engineered to be phosphatidyl inositol (PI) linked to a cell membrane, but can be removed from membranes by treatment with a phosphatidyl inositol cleaving enzyme, e.g., phosphatidyl inositol phospholipase-C. This releases the antigen in a biologically active form, and allows purification by standard procedures of protein chemistry. See, e.g., Low (1989) Biochim. Biophys.

Acta 988:427-454; Tse, et al. (1985) Science 230:1003-1008; and Brunner, et al. (1991) J. Cell Biol. 114:1275-1283.

Now that the IL-D110 and IL-D210 have been characterized, fragments or derivatives thereof can be prepared by conventional processes for synthesizing peptides. These include processes such as are described in Stewart and Young (1984) Solid Phase Peptide Synthesis, Pierce Chemical Co., Rockford, IL; Bodanszky and Bodanszky (1984) The Practice of Peptide Synthesis, Springer-Verlag, New York; Bodanszky (1984) The Principles of Peptide Synthesis, Springer-Verlag, New York; and Villafranca (ed. 1991) Techniques in Protein Chemistry II, Academic Press, San Diego, Ca.

VIII. Uses

The present invention provides reagents which will find use in diagnostic applications as described elsewhere herein, e.g., in IL-10 or IL-D10 mediated conditions, or below in the description of kits for diagnosis.

This invention also provides reagents with significant therapeutic potential. The IL-D10 (naturally occurring or recombinant), fragments thereof, and antibodies thereto, along with compounds identified as having binding affinity to IL-D10, should be useful in the treatment of conditions associated with abnormal physiology or development, including inflammatory conditions, either acute or chronic. In particular, modulation of physiology of lymphoid cells will be achieved by appropriate therapeutic treatment using the compositions provided herein. For example, a disease or disorder associated with abnormal expression or abnormal signaling by an IL-D10 should be a likely target for an agonist or antagonist. The new cytokine should play a role in regulation or development of hematopoietic cells, e.g., lymphoid or myeloid cells, which affect immunological responses, e.g., inflammation and/or autoimmune disorders.

In particular, the cytokine should mediate, in various contexts, cytokine synthesis by the cells, proliferation, etc.

Conversely, antagonists of IL-D110 or IL-D210, such as mutein variants of a naturally occurring form of an IL-D10 or

blocking antibodies, may provide a selective and powerful way to block immune responses, e.g., in situations as inflammatory or autoimmune responses, including rheumatoid arthritis, systemic lupus erythematosus (SLE), Hashimoto's autoimmune thyroiditis, as well as acute and chronic inflammatory responses, e.g., inflammatory bowel disease. See also Samter, et al. (eds.) Immunological Diseases vols. 1 and 2, Little, Brown and Co. Modulated cytokine release by the naturally occurring secreted form of IL-D10, which can be produced in large quantities by recombinant methods, or by blocking antibodies, should be regulatable by reagents made available herein, e.g., in a transplantation rejection situation.

In addition, certain combination compositions would be useful, e.g., with other modulators of inflammation. Such other molecules may include steroids, other versions of IL-10, including cellular species variants, or viral IL-10s, e.g., EBV or EHV, and all of their respective antagonists.

Various abnormal conditions are known in the various cell types which would produce IL-D110 or IL-D210 mRNA by Northern blot analysis. See Berkow (ed.) The Merck Manual of Diagnosis and Therapy, Merck & Co., Rahway, N.J.; Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y.; and Weatherall, et al. (eds.) Oxford Textbook of Medicine, Oxford University Press, Oxford. Many other medical conditions and diseases involve activation by macrophages or monocytes, and many of these will be responsive to treatment by an agonist or antagonist provided herein. See, e.g., Stites and Terr (eds.; 1991) Basic and Clinical Immunology Appleton and Lange, Norwalk, Connecticut; and Samter, et al. (eds.) Immunological Diseases Little, Brown and Co. These problems should be susceptible to prevention or treatment using compositions provided herein.

IL-D110 or IL-D210 antibodies can be purified and then administered to a patient, veterinary or human. These reagents can be combined for therapeutic use with additional active or inert ingredients, e.g., in conventional pharmaceutically acceptable carriers or diluents, e.g., immunogenic adjuvants, along with physiologically innocuous

stabilizers, excipients, or preservatives. These combinations can be sterile filtered and placed into dosage forms as by lyophilization in dosage vials or storage in stabilized aqueous preparations. This invention also contemplates use of antibodies or binding fragments thereof, including forms which are not complement binding.

Drug screening using IL-D10 or fragments thereof can be performed to identify compounds having binding affinity to or other relevant biological effects on IL-D10 functions, including isolation of associated components. Subsequent biological assays can then be utilized to determine if the compound has intrinsic stimulating activity and is therefore a blocker or antagonist in that it blocks the activity of the cytokine. Likewise, a compound having intrinsic stimulating activity can activate the signal pathway and is thus an agonist in that it simulates the activity of an IL-D10. This invention further contemplates the therapeutic use of blocking antibodies to either IL-D10 as antagonists and of stimulatory antibodies as agonists. This approach should be particularly useful with other IL-D10 species variants.

The cytokines, or antagonists, may be useful in anti-tumor therapy. The viral correlation may suggest that the cytokine may be important in viral infection or proliferation processes, or oncology processes, e.g., oncogenic transformation and proliferative conditions, as cancers or leukemias. See, e.g., Thorn, et al. Harrison's Principles of Internal Medicine, McGraw-Hill, N.Y.

The quantities of reagents necessary for effective therapy will depend upon many different factors, including means of administration, target site, physiological state of the patient, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds. 1990) Goodman and

Gilman's: The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn. Methods for administration are discussed therein and below, e.g., for oral, intravenous, intraperitoneal, or intramuscular administration, transdermal diffusion, and others. Pharmaceutically acceptable carriers will include water, saline, buffers, and other compounds described, e.g., in the Merck Index, Merck & Co., Rahway, New Jersey. Dosage ranges would ordinarily be expected to be in amounts lower than 1 mM concentrations, typically less than about 10 μM concentrations, usually less than about 100 nM, preferably less than about 10 pM (picomolar), and most preferably less than about 1 fM (femtomolar), with an appropriate carrier. Slow release formulations, or a slow release apparatus will often be utilized for continuous or long term administration. See, e.g., Langer (1990) Science 249:1527-1533.

IL-D110 and IL-D210, fragments thereof, and antibodies to it or its fragments, antagonists, and agonists, may be administered directly to the host to be treated or, depending on the size of the compounds, it may be desirable to conjugate them to carrier proteins such as ovalbumin or serum albumin prior to their administration. Therapeutic formulations may be administered in many conventional dosage formulations. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a pharmaceutical formulation. Formulations typically comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof. Each carrier should be both pharmaceutically and physiologically acceptable in the sense of being compatible with the other ingredients and not injurious to the patient. Formulations include those suitable for oral, rectal, nasal, topical, or parenteral (including subcutaneous, intramuscular, intravenous and intradermal) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. See, e.g., Gilman, et al. (eds. 1990) Goodman and Gilman's:

The Pharmacological Bases of Therapeutics, 8th Ed., Pergamon Press; and Remington's Pharmaceutical Sciences, 17th ed. (1990), Mack Publishing Co., Easton, Penn.; Avis, et al. (eds. 1993) Pharmaceutical Dosage Forms: Parenteral Medications, Dekker, New York; Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Tablets, Dekker, New York; and Lieberman, et al. (eds. 1990) Pharmaceutical Dosage Forms: Disperse Systems, Dekker, New York. The therapy of this invention may be combined with or used in association with other agents, e.g., other types of IL-10s, or their respective antagonists.

Both the naturally occurring and the recombinant form of the IL-D10s of this invention are particularly useful in kits and assay methods which are capable of screening compounds for binding activity to the proteins. Several methods of automating assays have been developed in recent years so as to permit screening of tens of thousands of compounds in a short period. See, e.g., Fodor, et al. (1991) Science 251:767-773, which describes means for testing of binding affinity by a plurality of defined polymers synthesized on a solid substrate. The development of suitable assays can be greatly facilitated by the availability of large amounts of purified, soluble IL-D110 or IL-D210 as provided by this invention.

Other methods can be used to determine the critical residues in the cytokine receptor interactions. Mutational analysis can be performed, e.g., see Somoza, et al. (1993) J. Exptl. Med. 178:549-558, to determine specific residues critical in the interaction and/or signaling.

For example, antagonists can normally be found once the antigen has been structurally defined, e.g., by tertiary structure data. Testing of potential interacting analogs is now possible upon the development of highly automated assay methods using a purified IL-D10. In particular, new agonists and antagonists will be discovered by using screening techniques described herein. Of particular importance are compounds found to have a combined binding affinity for a spectrum of IL-D10 molecules, e.g., compounds which can serve as antagonists for species variants of IL-D10.

One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant DNA molecules expressing an IL-D10. Cells may be isolated which express an IL-D10 in isolation from other molecules. Such cells, either in viable or fixed form, can be used for standard binding partner binding assays. See also, Parce, et al. (1989) Science 246:243-247; and Owicki, et al. (1990) Proc. Nat'l Acad. Sci. USA 87:4007-4011, which describe sensitive methods to detect cellular responses.

Another technique for drug screening involves an approach which provides high throughput screening for compounds having suitable binding affinity to an IL-D10 and is described in detail in Geysen, European Patent Application 84/03564, published on September 13, 1984. First, large numbers of different small peptide test compounds are synthesized on a solid substrate, e.g., plastic pins or some other appropriate surface, see Fodor, et al. (1991). Then all the pins are reacted with solubilized, unpurified or solubilized, purified IL-D10, and washed. The next step involves detecting bound IL-D10.

Rational drug design may also be based upon structural studies of the molecular shapes of the IL-D10 and other effectors or analogs. Effectors may be other proteins which mediate other functions in response to binding, or other proteins which normally interact with IL-D10, e.g., a receptor. One means for determining which sites interact with specific other proteins is a physical structure determination, e.g., x-ray crystallography or 2 dimensional NMR techniques. These will provide guidance as to which amino acid residues form molecular contact regions, as modeled, e.g., against cellular IL-10. For a detailed description of protein structural determination, see, e.g., Blundell and Johnson (1976) Protein Crystallography, Academic Press, New York.

IX. Kits

This invention also contemplates use of IL-110 or IL-210 proteins, fragments thereof, peptides, and their fusion products in a variety of diagnostic kits and methods for

detecting the presence of another IL-D10 or binding partner. Typically the kit will have a compartment containing either a defined IL-D10 peptide or gene segment or a reagent which recognizes one or the other, e.g., IL-D10 fragments or antibodies.

A kit for determining the binding affinity of a test compound to an IL-D10 would typically comprise a test compound; a labeled compound, for example a binding partner or antibody having known binding affinity for IL-D10; a source of IL-D10 (naturally occurring or recombinant); and a means for separating bound from free labeled compound, such as a solid phase for immobilizing the molecule. Once compounds are screened, those having suitable binding affinity to the antigen can be evaluated in suitable biological assays, as are well known in the art, to determine whether they act as agonists or antagonists to the IL-D10 signaling pathway. The availability of recombinant IL-D10 polypeptides also provide well defined standards for calibrating such assays.

A preferred kit for determining the concentration of, e.g., an IL-D10 in a sample would typically comprise a labeled compound, e.g., binding partner or antibody, having known binding affinity for the antigen, a source of cytokine (naturally occurring or recombinant) and a means for separating the bound from free labeled compound, e.g., a solid phase for immobilizing the IL-D10. Compartments containing reagents, and instructions, will normally be provided.

Antibodies, including antigen binding fragments, specific for the IL-D110 or IL-D210 or fragments are useful in diagnostic applications to detect the presence of elevated levels of IL-D10 and/or its fragments. Such diagnostic assays can employ lysates, live cells, fixed cells, immunofluorescence, cell cultures, body fluids, and further can involve the detection of antigens related to the antigen in serum, or the like. Diagnostic assays may be homogeneous (without a separation step between free reagent and antigen-binding partner complex) or heterogeneous (with a separation step). Various commercial assays exist, such as radioimmunoassay (RIA), enzyme-linked immunosorbent assay

(ELISA), enzyme immunoassay (EIA), enzyme-multiplied immunoassay technique (EMIT), substrate-labeled fluorescent immunoassay (SLFIA), and the like. See, e.g., Van Vunakis, et al. (1980) Meth Enzymol. 70:1-525; Harlow and Lane (1980) Antibodies: A Laboratory Manual, CSH Press, NY; and Coligan, et al. (eds. 1993) Current Protocols in Immunology, Greene and Wiley, NY.

Anti-idiotypic antibodies may have similar use to diagnose presence of antibodies against an IL-D10, as such may be diagnostic of various abnormal states. For example, overproduction of IL-D10 may result in production of various immunological reactions which may be diagnostic of abnormal physiological states, particularly in proliferative cell conditions such as cancer or abnormal activation or differentiation.

Frequently, the reagents for diagnostic assays are supplied in kits, so as to optimize the sensitivity of the assay. For the subject invention, depending upon the nature of the assay, the protocol, and the label, either labeled or unlabeled antibody or binding partner, or labeled IL-D10 is provided. This is usually in conjunction with other additives, such as buffers, stabilizers, materials necessary for signal production such as substrates for enzymes, and the like. Preferably, the kit will also contain instructions for proper use and disposal of the contents after use. Typically the kit has compartments for each useful reagent. Desirably, the reagents are provided as a dry lyophilized powder, where the reagents may be reconstituted in an aqueous medium providing appropriate concentrations of reagents for performing the assay.

Many of the aforementioned constituents of the drug screening and the diagnostic assays may be used without modification or may be modified in a variety of ways. For example, labeling may be achieved by covalently or non-covalently joining a moiety which directly or indirectly provides a detectable signal. In many of these assays, the binding partner, test compound, IL-D10, or antibodies thereto can be labeled either directly or indirectly. Possibilities

for direct labeling include label groups: radiolabels such as ^{125}I, enzymes (U.S. Pat. No. 3,645,090) such as peroxidase and alkaline phosphatase, and fluorescent labels (U.S. Pat. No. 3,940,475) capable of monitoring the change in fluorescence intensity, wavelength shift, or fluorescence polarization. Possibilities for indirect labeling include biotinylation of one constituent followed by binding to avidin coupled to one of the above label groups.

There are also numerous methods of separating the bound from the free IL-D10, or alternatively the bound from the free test compound. The IL-D10 can be immobilized on various matrixes followed by washing. Suitable matrixes include plastic such as an ELISA plate, filters, and beads. See, e.g., Coligan, et al. (eds. 1993) Current Protocols in Immunology, Vol. 1, Chapter 2, Greene and Wiley, NY. Other suitable separation techniques include, without limitation, the fluorescein antibody magnetizable particle method described in Rattle, et al. (1984) Clin. Chem. 30:1457-1461, and the double antibody magnetic particle separation as described in U.S. Pat. No. 4,659,678.

Methods for linking proteins or their fragments to the various labels have been extensively reported in the literature and do not require detailed discussion here. Many of the techniques involve the use of activated carboxyl groups either through the use of carbodiimide or active esters to form peptide bonds, the formation of thioethers by reaction of a mercapto group with an activated halogen such as chloroacetyl, or an activated olefin such as maleimide, for linkage, or the like. Fusion proteins will also find use in these applications.

Another diagnostic aspect of this invention involves use of oligonucleotide or polynucleotide sequences taken from the sequence of an IL-D10. These sequences can be used as probes for detecting levels of the IL-D10 message in samples from patients suspected of having an abnormal condition, e.g., inflammatory or autoimmune. Since the cytokine may be a marker or mediator for activation, it may be useful to determine the numbers of activated cells to determine, e.g.,

when additional therapy may be called for, e.g., in a preventative fashion before the effects become and progress to significance. The preparation of both RNA and DNA nucleotide sequences, the labeling of the sequences, and the preferred size of the sequences has received ample description and discussion in the literature. See, e.g., Langer-Safer, et al. (1982) Proc. Nat'l. Acad. Sci. 79:4381-4385; Caskey (1987) Science 236:962-967; and Wilchek et al. (1988) Anal. Biochem. 171:1-32.

Diagnostic kits which also test for the qualitative or quantitative expression of other molecules are also contemplated. Diagnosis or prognosis may depend on the combination of multiple indications used as markers. Thus, kits may test for combinations of markers. See, e.g., Viallet, et al. (1989) Progress in Growth Factor Res. 1:89-97. Other kits may be used to evaluate other cell subsets.

X. Isolating the IL-D110 or IL-D210 Receptor

Having isolated a ligand of a specific ligand-receptor interaction, methods exist for isolating the receptor. See, Gearing, et al. (1989) EMBO J. 8:3667-3676. For example, means to label the IL-D10 cytokine without interfering with the binding to its receptor can be determined. For example, an affinity label can be fused to either the amino- or carboxyl-terminus of the ligand, though based on IL-10, the amino-terminus is more likely to succeed. Such label may be a FLAG epitope tag, or, e.g., an Ig or Fc domain. An expression library can be screened for specific binding of the cytokine, e.g., by cell sorting, or other screening to detect subpopulations which express such a binding component. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90:11267-11271; and Liu, et al. (1994) J. Immunol. 152:1821-29. Alternatively, a panning method may be used. See, e.g., Seed and Aruffo (1987) Proc. Nat'l Acad. Sci. USA 84:3365-3369.

Protein cross-linking techniques with label can be applied to isolate binding partners of the IL-D10 cytokine. This would allow identification of proteins which specifically

interact with the cytokine, e.g., in a ligand-receptor like manner.

Early experiments will be performed to determine whether the known IL-10R is involved in response(s) to IL-D10. It is also quite possible that the functional IL-10 receptor complex may share many or all components with an IL-D10 receptor complex, either a specific receptor subunit or an accessory receptor subunit.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

EXAMPLES

General Methods

Some of the standard methods are described or referenced, e.g., in Maniatis, et al. (1982) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor Press; Sambrook, et al. (1989) Molecular Cloning: A Laboratory Manual (2d ed.), vols. 1-3, CSH Press, NY; Ausubel, et al., Biology, Greene Publishing Associates, Brooklyn, NY; or Ausubel, et al. (1987 and Supplements) Current Protocols in Molecular Biology, Greene and Wiley, New York; Innis, et al. (eds.)(1990) PCR Protocols: A Guide to Methods and Applications, Academic Press, N.Y. Methods for protein purification include such methods as ammonium sulfate precipitation, column chromatography, electrophoresis, centrifugation, crystallization, and others. See, e.g., Ausubel, et al. (1987 and periodic supplements); Deutscher (1990) "Guide to Protein Purification" in Methods in Enzymology vol. 182, and other volumes in this series; and manufacturer's literature on use of protein purification products, e.g., Pharmacia, Piscataway, N.J., or Bio-Rad, Richmond, CA. Combination with recombinant techniques allow fusion to appropriate segments, e.g., to a FLAG sequence or an

equivalent which can be fused via a protease-removable sequence. See, e.g., Hochuli (1989) Chemische Industrie 12:69-70; Hochuli (1990) "Purification of Recombinant Proteins with Metal Chelate Absorbent" in Setlow (ed.) Genetic Engineering, Principle and Methods 12:87-98, Plenum Press, N.Y.; and Crowe, et al. (1992) QIAexpress: The High Level Expression & Protein Purification System QUIAGEN, Inc., Chatsworth, CA. Cell culture techniques are described in Doyle, et al. (eds. 1994) Cell and Tissue Culture: Laboratory Procedures, John Wiley and Sons, NY.

FACS analyses are described in Melamed, et al. (1990) Flow Cytometry and Sorting Wiley-Liss, Inc., New York, NY; Shapiro (1988) Practical Flow Cytometry Liss, New York, NY; and Robinson, et al. (1993) Handbook of Flow Cytometry Methods Wiley-Liss, New York, NY. Fluorescent labeling of appropriate reagents was performed by standard methods.

EXAMPLE 1: Identification of Human IL-D110 or IL-D210

Motifs characteristic of mammalian IL-10s were identified, recognizing a number of specific IL-10 homologs found among different species. Screening various sequence databases for these features identified two distinct sequences possessing the motifs. These sequences were extended out to provide the more complete primate homologs. These are designated IL-D110 (for DNAX designation 110 and 210, both being related to IL-10). The term "IL-D10" will refer collectively to the two. Applicants have used unique DNAX identification numbers to avoid confusion with labels not generally accepted by existing nomenclature groups representing the scientific community.

PCR products are cloned using, e.g., a TA cloning kit (Invitrogen). The resulting cDNA plasmids are resequenced from both termini on an automated sequencer (Applied Biosystems) to confirm.

EXAMPLE 2: Cellular Expression of Human IL-D110 or IL-D210

Because of the sequence similarity to human IL-10, distribution will be investigated for similar type cell types.

A probe specific for cDNA encoding primate IL-D110 or IL-D210 is labeled, e.g., by random priming.

Using a commercial dot spot mRNA hybridization filter and standard hybridization conditions, expression is evaluated in a wide spectrum of cell types and species. Expression data from RT-PCR and from the mRNA-dot hybridization should be confirmed by sensitive Northern or other means.

EXAMPLE 3: Chromosome mapping of Human IL-D110 or IL-D210

Chromosome mapping is a standard technique. See, e.g., BIOS Laboratories (New Haven, CT) and methods for using a mouse somatic cell hybrid panel with PCR.

EXAMPLE 4: Purification of IL-10 homolog Protein

Multiple transfected cell lines are screened for one which expresses the cytokine at a high level compared with other cells. Various cell lines are screened and selected for their favorable properties in handling. Natural IL-D10 can be isolated from natural sources, or by expression from a transformed cell using an appropriate expression vector. Early results suggest that the cytokine, after secretion, rebinds to the cell surface. Purification of the expressed protein is achieved by standard procedures, or may be combined with engineered means for effective purification at high efficiency from cell lysates or supernatants. FLAG or His_6 segments can be used for such purification features. Alternatively, affinity chromatography may be used with specific antibodies, see below.

EXAMPLE 5: Isolation of Homologous IL-D10 Genes

The IL-D110 or IL-D210 cDNA can be used as a hybridization probe to screen a library from a desired source, e.g., a primate cell cDNA library. Many different species can be screened both for stringency necessary for easy hybridization, and for presence using a probe. Appropriate hybridization conditions will be used to select for clones exhibiting specificity of cross hybridization.

Screening by hybridization using degenerate probes based upon the peptide sequences will also allow isolation of appropriate clones. Alternatively, use of appropriate primers for PCR screening will yield enrichment of appropriate nucleic acid clones.

Similar methods are applicable to isolate either species, polymorphic, or allelic variants. Species variants are isolated using cross-species hybridization techniques based upon isolation of a full length isolate or fragment from one species as a probe.

Alternatively, antibodies raised against human IL-D10 will be used to screen for cells which express cross-reactive proteins from an appropriate, e.g., cDNA library. The purified protein or defined peptides are useful for generating antibodies by standard methods, as described above. Synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. The resulting antibodies are used for screening, purification, or diagnosis, as described.

EXAMPLE 6: Preparation of antibodies specific for IL-D10

Synthetic peptides or purified protein are presented to an immune system to generate monoclonal or polyclonal antibodies. See, e.g., Coligan (1991) Current Protocols in Immunology Wiley/Greene; and Harlow and Lane (1989) Antibodies: A Laboratory Manual Cold Spring Harbor Press. Polyclonal serum, or hybridomas may be prepared. In appropriate situations, the binding reagent is either labeled as described above, e.g., fluorescence or otherwise, or immobilized to a substrate for panning methods.

EXAMPLE 7: Evaluation of Breadth of Biological Functions

The native, recombinant, and fusion proteins would be tested for agonist and antagonist activity in many biological assay systems, e.g., on T-cells, B-cells, NK, macrophages, dendritic cells, hematopoietic progenitors, etc. Because of

the IL-10 structural relationship, assays related to IL-10 activity would analyzed

IL-D10s are evaluated for agonist or antagonist activity on transfected cells expressing IL-10 receptor and controls. See, e.g., Ho, et al. (1993) Proc. Nat'l Acad. Sci. USA 90, 11267-11271; Ho, et al. (1995) Mol. Cell. Biol. 15:5043-5053;and Liu, et al. (1994). J. Immunol. 152:1821-1829.

Based, in part, upon the structural homology to IL-10, the IL-D10s are evaluated for effect in macrophage/dendritic cell activation and antigen presentation assays, T cell cytokine production and proliferation in response to antigen or allogeneic stimulus. See, e.g., de Waal Malefyt et al. (1991) J. Exp. Med. 174:1209-1220; de Waal Malefyt et al. (1991) J. Exp. Med. 174:915-924; Fiorentino, et al. (1991) J. Immunol. 147, 3815-3822; Fiorentino, et al. (1991) J. Immunol. 146:3444-3451; and Groux, et al. (1996) J. Exp. Med. 184:19-29.

IL-D10s will also be evaluated for effects on NK cell stimulation. Assays may be based, e.g., on Hsu, et al. (1992) Internat. Immunol. 4:563-569; and Schwarz, et al. (1994) J. Immunother. 16:95-104.

B cell growth and differentiation effects will be analyzed, e.g., by the methodology described, e.g., in Defrance, et al. (1992). J. Exp. Med. 175:671-682; Rousset, et al (1992) Proc. Nat'l Acad. Sci. USA 89:1890-1893; including IgG2 and IgA2 switch factor assays. Note that, unlike COS7 supernatants, NIH3T3 and COP supernatants apparently do not interfere with human B cell assays.

EXAMPLE 8: IL-D110 autocrine signaling

This research was initiated to define biological activities of IL-10 related molecules. Based on the publication by Dumoutier, et al. (2000) J. Immunol. 164:1814 - 1819, which describes phosphorylation of STAT-3 and STAT-5 signal transducing molecules in response to IL-TIFα by MES13 (ATCC CRL 1927) and PC12 (ATCC CRL 1721) cells, but not by RAW264 and peritoneal cells, the activity of human CP-2 on human mesangial cells was tested. MES13 is a murine kidney

mesangial cell line and PC12 is a rat pheochromocytoma cell line, IL-TIFα is a murine homolog of the human cytokine IL-D110.

Human primary mesangial cells were obtained from Clonetics (San Diego, CA) and cultured according to the suppliers' recommendations in MsGM (BioWhittaker) with or without human IL-D110 for 2, 6, and 12 h. mRNA was isolated from these cultures, reverse transcribed, and the cDNA analyzed for expression of IL-10 homologs including IL-D110. IL-D110 induced the expression of IL-D110 mRNA in human mesangial cells. IL-D110 mRNA was induced by 1000 fold at 12 h. This induction was time and dose dependent. In addition, it was confirmed that human IL-D110 induced phosphorylation of STAT-3 by human mesangial cells.

These results indicate that human IL-D110 is biologically active and can induce biological responses in human mesangial cells. This biological response includes the induction of STAT-3 phosphorylation and the generation of an autocrine positive regulatory mechanism of expression. STAT-3 phosphorylation is associated with the induction of biological responses to many cytokines, and has been shown to be important for proliferation and differentiation of cells responsive to cytokine growth factors. It can thus be expected that IL-D110 will affect processes that influence the proliferation and function of mesangial cells. This could lead to a better understanding of the role of mesangial cells in the kidney and may be important to diseases that affect clearance of metabolites and the ionic balance of blood. In addition, IL-D110 may affect immune related complications through its effects on mesangial cells, notably affecting inflammatory responses in glomerulonephritis, etc.

All references cited herein are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

Many modifications and variations of this invention can be made without departing from its spirit and scope, as will

be apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims, along with the full scope of equivalents to which such claims are entitled.

WHAT IS CLAIMED IS:

1. An isolated or recombinant polynucleotide encoding an antigenic polypeptide comprising at least 17 contiguous amino acids from the mature polypeptide from SEQ ID NO: 2.

2. The polynucleotide of Claim 1, encoding a mature polypeptide from SEQ ID NO: 2.

3. The polynucleotide of Claim 1, which hybridizes at 55° C, less than 500 mM salt, and 50% formamide to the coding portions of SEQ ID NO: 1.

4. The polynucleotide of Claim 3:

a) wherein said temperature is at least 65° C;

b) wherein said salt is less than 200 mM;

c) wherein said temperature is at least 60° C and said salt is less than 300 mM; or

d) comprising at least 35 contiguous nucleotides of the coding portion of SEQ ID NO: 1.

5. An expression vector comprising said polynucleotide of Claim 1.

6. A host cell containing said expression vector of Claim 5, including a eukaryotic cell.

7. A method of making an antigenic polypeptide comprising expressing a recombinant polynucleotide of Claim 1.

8. A method for forming a duplex with a polynucleotide of Claim 1, comprising contacting said polynucleotide with a probe that hybridizes, under stringent conditions, to at least 25 contiguous nucleotides of the coding portion of SEQ ID NO: 1; thereby forming said duplex.

9. A kit for the detection of a polynucleotide of Claim 1, comprising a polynucleotide that hybridizes, under

stringent hybridization conditions, to at least 17 contiguous nucleotides of a polynucleotide of Claim 1.

10. The kit of claim 9, wherein said probe is detectably labeled.

11. A binding compound comprising an antibody binding site which specifically binds to:

a) at least 17 contiguous amino acids from SEQ ID NO: 2; or

b) a mature polypeptide from SEQ ID NO: 2.

12. The binding compound of Claim 11, wherein:

a) said antibody binding site is:

1) specifically immunoreactive with a polypeptide of SEQ ID NO: 2;

2) raised against a purified or recombinantly produced human IL-D110 protein; or

3) in a monoclonal antibody, Fab, or F(ab)2; or

b) said binding compound is:

1) an antibody molecule;

2) a polyclonal antiserum;

3) detectably labeled;

4) sterile; or

5) in a buffered composition.

13. A method using the binding compound of Claim 11, comprising contacting said binding compound with a biological sample comprising an antigen, wherein said contacting results in formation of a binding compound:antigen complex.

14. The method of Claim 13, wherein said biological sample is from a human, and wherein said binding compound is an antibody.

15. A detection kit comprising said binding compound of Claim 12, and:

a) instructional material for the use of said binding compound for said detection; or

b) a compartment providing segregation of said binding compound.

16. A substantially pure or isolated antigenic polypeptide, which binds to said binding composition of Claim 11, and further comprises at least 17 contiguous amino acids from SEQ ID NO: 2.

17. The polypeptide of Claim 16, which:

a) comprises at least a fragment of at least 17 contiguous amino acid residues from a primate IL-D110 polypeptide;

b) is a soluble polypeptide;

c) is detectably labeled;

d) is in a sterile composition;

e) is in a buffered composition;

f) binds to a cell surface receptor;

g) is recombinantly produced; or

h) has a naturally occurring polypeptide sequence.

18. The polypeptide of Claim 17, which comprises at least 25 contiguous amino acids of SEQ ID NO: 2.

19. A method of modulating physiology or development of a cell or tissue culture cells comprising contacting said cell with an agonist or antagonist of a primate IL-D110.

20. The method of Claim 19, wherein:

a) said agonist is a mutein of said primate IL-D110;

b) said antagonist is an antibody which binds to said primate IL-D110;

c) said cell is a mesangial cell; or

d) said modulating is inducing production of IL-D110 and said contacting is with said agonist.

SEQUENCE SUBMISSION

SEQ ID NO: 1 is primate IL-D110 natural nucleic acid sequence.
SEQ ID NO: 2 is primate IL-D110 natural amino acid sequence.
SEQ ID NO: 3 is primate IL-D110 reverse translation.
SEQ ID NO: 4 is primate IL-D210 partial natural nucleic acid sequence.
SEQ ID NO: 5 is primate IL-D210 partial amino acid sequence.
SEQ ID NO: 6 is primate IL-D210 reverse translation.
SEQ ID NO: 7 is primate IL-10 amino acid sequence.
SEQ ID NO: 8 is primate AK155 amino acid sequence.
SEQ ID NO: 9 is primate IL-D19 amino acid sequence.
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gct gcg ccc atc agc tcc cac tgc agg ctt gac aag tcc aac ttc cag   202
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cac gga gtc agt atg agt gag cgc tgc tat ctg atg aag cag gtg ctg   346
His Gly Val Ser Met Ser Glu Arg Cys Tyr Leu Met Lys Gln Val Leu
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aac ttc acc ctt gaa gaa gtg ctg ttc cct caa tct gat agg ttc cag   394
Asn Phe Thr Leu Glu Glu Val Leu Phe Pro Gln Ser Asp Arg Phe Gln
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cct tat atg cag gag gtg gtg ccc ttc ctg gcc agg ctc agc aac agg   442
Pro Tyr Met Gln Glu Val Val Pro Phe Leu Ala Arg Leu Ser Asn Arg
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cta agc aca tgt cat att gaa ggt gat gac ctg cat atc cag agg aat   490
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Pro Tyr Met Gln Glu Val Val Pro Phe Leu Ala Arg Leu Ser Asn Arg
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mgnytngaya arwsnaaytt ycarcarccn tayathacna aymgnacntt yatgytngcn 180

aargargcnw snytngcnga yaayaayacn gaygtnmgny tnathggnga raarytntty 240

cayggngtnw snatgwsnga rmgntgytay ytnatgaarc argtnytnaa yttyacnytn 300

gargargtny tnttyccnca rwsngaymgn ttycarccnt ayatgcarga rgtngtnccn 360

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athcarmgna aygtncaraa rytnaargay acngtnaara arytnggnga rwsnggngar 480

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ctc cgg ctc tgt cat gcc cac atg aca tgc cat tgt ggg gag gaa gca   191
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Lys Ala Leu Gly Glu Val Asp Ile Leu Leu Thr Trp Met Gln Lys Phe
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Tyr Lys Leu

INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/US 00/14729

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/19 C12N15/24 C07K14/52 C07K14/54 C12Q1/68
C07K16/24 G01N33/68 A61K38/19 A61K38/20 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, STRAND, PAJ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MOORE K W ET AL: "HOMOLOGY OF CYTOKINE SYNTHESIS INHIBITORY FACTOR (IL-10) TO THE EPSTEIN-BARR VIRUS GENE BCRFI" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 248, no. 4960, 8 June 1990 (1990-06-08), pages 1230-1234, XP000255849 ISSN: 0036-8075 the whole document	1-20

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2000	29/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Le Cornec, N

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Form PCT/ISA/210 (second sheet) (July 1992)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claim 19 refers to an agonist or antagonist of primate iL-D110 and the search has been done in view of claim 20 defining said agonist and antagonist.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14729

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL AC007458 'Online! accession number AC007458, 5 May 1999 (1999-05-05) D. MUZNY ET AL: "Homo sapiens 12q15 BAC RPCI11-444B24 complete sequence" XP002147212 "99,2 % identity in 623 nt overlap with sequence ID no.1 from nt1140-nt517. reverse orientation" abstract	9
X	DATABASE EMBL 'Online! AQ104025 accession number AQ104025, 4 September 1998 (1998-09-04) G.G. MAHAIRAS ET AL: "sequence tagged connectors: A sequence approach to mapping and scanning the human genome" XP002147213 abstract & G.G. MAHAIRAS ET AL: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 96, no. 17, 1999, pages 9739-9744, NATIONAL ACADEMY OF SCIENCE. WASHINGTON., US ISSN: 0027-8424	8,9
A	EP 0 405 980 A (SCHERING CORP) 2 January 1991 (1991-01-02) claims	1-20
P,X	DATABASE EMBL CAB75546 'Online! accession number CAB75546, 21 March 2000 (2000-03-21) L. DUMOUTIER ET AL: "Cloning and characterization of iL-10-related-T cell-derived inducible factor (iL-TIF), a novel cytokine structurally related to iL-10 and inducible by iL-9" XP002147214 abstract -& L. DUMOUTIER ET AL: JOURNAL OF IMMUNOLOGY., vol. 164, February 2000 (2000-02), pages 1814-1819, XP002147210 ISSN: 0019-2805	1-20

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/14729

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	DATABASE EMBL CAC06085 'Online! accession number CAC06085, 1 September 2000 (2000-09-01) L. DUMOUTIER ET AL: "Human interleukin-10-related T cell -derived inducible factor: Molecular cloning and functional characterization as an hepatocyte-stimulating factor " XP002147215 abstract -& L. DUMOUTIER ET AL: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA., vol. 97, no. 18, August 2000 (2000-08), pages 10144-10149, XP002147211 WASHINGTON US	1-20

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int: ional Application No
PCT/US 00/14729

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0405980 A	02-01-1991	AT 180833 T	15-06-1999
		AU 635058 B	11-03-1993
		AU 6077090 A	17-01-1991
		CA 2062763 A	29-12-1990
		CN 1051393 A	15-05-1991
		CS 9104145 A	16-12-1992
		DE 69033143 D	08-07-1999
		DE 69033143 T	21-10-1999
		EP 0567450 A	03-11-1993
		ES 2132068 T	16-08-1999
		GR 3030404 T	30-09-1999
		HU 216310 B	28-06-1999
		JP 2813063 B	22-10-1998
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		KR 183035 B	01-04-1999
		MX 9203318 A	01-07-1992
		NO 301718 B	01-12-1997
		NZ 234291 A	25-11-1992
		PT 94514 A,B	18-04-1991
		SG 52282 A	28-09-1998
		WO 9100349 A	10-01-1991
		US 5231012 A	27-07-1993
		ZA 9005060 A	27-03-1991